

Applicant : Terry B. Strom et al.
Serial No. : 09/855,313
Filed : May 14, 2001
Page : 4

Attorney's Docket No.: 01948-056001

REMARKS

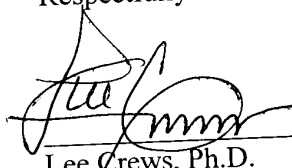
Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: November 28 2001



Lee Crews, Ph.D.
Reg. No. 43,567

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 21, line 26, has been amended as follows:

For the construction of the plasmid FLAG-HMK-IL-15, a 322 bp cDNA fragment encoding mature IL-15 protein was amplified by PCR utilizing synthetic oligonucleotides [sense 5'-GGAATTCAACTGGGTGAATGTAATA-3' (SEQ ID NO:5; *EcoRI* site (underlined) plus bases 145-162); antisense 5'-CGGGATCCTCAAGAAGTGTGATGAA-3' (SEQ ID NO:[5] 6; *BamHI* site [underlined] plus bases 472-489)]. The template DNA was obtained from PHA-activated human PBMCs. The PCR product was purified, digested with *EcoRI* and *BamHI*, and cloned into the pAR(DRI)59/60 plasmid digested with *EcoRI*-*BamHI* as described (Blonar *et al.*, *Science* 256:1014, 1992; LeClair *et al.*, *Proc. Natl. Acad. Sci. USA* 89:8145, 1992). The backbone of the pAR(DRI)59/60 plasmid contains in frame sequences encoding the FLAG and HMK recognition peptide sequences (Blonar *et al.*, *Science* 256:1014, 1992; LeClair *et al.*, *Proc. Natl. Acad. Sci. USA* 89:8145, 1992).

Paragraph beginning at page 25, line 14, has been amended as follows:

The human IL-15 protein bearing a double mutation (Q149D; Q156D) was designed to target the putative sites critical for binding to the IL-2R subunit. The polar, but uncharged glutamine residues at positions 149 and 156 were mutated into acidic residues of aspartic acid utilizing PCR-assisted mutagenesis. A cDNA encoding the double mutant of IL-15 was amplified by PCR utilizing a synthetic sense oligonucleotide [5'-GGAATTCAACTGGGTGAATGTAATA-3' (SEQ ID NO:[] 5); *EcoRI* site (underlined hexamer) plus bases 145-162] and a synthetic antisense oligonucleotide (5'-CGGGATCCTCAAGAAGTGTGATGAACATGTCGACAAT-ATGTACAAAAGTGTCCAAAAT-3' (SEQ ID NO:[] 7); *BamHI* site (underlined hexamer) plus bases 438-489; mutated bases are singly underlined]. The template was a plasmid containing cDNA that encodes human FLAG-HMK-IL-15. The amplified fragment was digested with *EcoRI*/*BamHI* and cloned into the pAR(DRI)59/60 plasmid digested with

Applicant : Terry B. Strom et al.
Serial No. : 09/855,313
Filed : May 14, 2001
Page : 6

Attorney's Docket No.: 01948-056001

EcoRI/BamRI as described (LeClair *et al.*, *Proc. Natl. Acad. Sci. USA* 89:8145, 1989). The presence of a mutation at residue 156 was confirmed by digestion with *Sall*; the mutation introduces a new *Sall* restriction site. In addition, mutations were verified by DNA sequencing, according to standard techniques. The FLAG-HMK-IL-15 (Q149D; Q156D) double mutant protein was produced, purified, and verified by sequencing as described above for the FLAG-HMK-IL-15 wild-type protein.